

Listing of the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1 - 9. (Cancelled)

10. (Currently amended) A method for the quantification of one or more target nucleic acid molecules in a sample comprising hybridizing one or more detectably labeled oligonucleotides with said one or more target nucleic acid molecules, and quantifying the amount of said one or more target nucleic acid molecules;

wherein said one or more oligonucleotides comprise one or more detectable labels located only internally and said one or more labels undergo a detectable change in an observable property upon said hybridizing ~~to said one or more target nucleic acid molecules, and quantifying the amount of said one or more target nucleic acid molecules; and~~

wherein said detectable change ~~in an observable property~~ is not the result of a ~~transfer of energy between two different compounds attached to said one or more oligonucleotides~~ fluorescence resonance energy transfer (FRET).

11. (Currently amended) A method for the quantitation or detection of one or more target nucleic acid molecules in a sample during nucleic acid synthesis comprising:
mixing one or more a target nucleic acid molecules with one or more detectably labeled oligonucleotides, wherein said one or more oligonucleotides comprise one or more

detectable labels located only internally and said one or more labels undergo a detectable change in an observable property upon ~~hybridizing~~ hybridization of said one or more oligonucleotides to said one or more target nucleic acid molecules; wherein said detectable change ~~in an observable property~~ is not the result of ~~a transfer of energy between two different compounds attached to said one or more oligonucleotides~~ fluorescent resonance energy transfer (FRET);

incubating said mixture under conditions sufficient to synthesize one or more nucleic acid molecules complementary to all or a portion of said one or more target nucleic acid molecules, said one or more synthesized nucleic acid molecules comprising said one or more oligonucleotides; and

detecting the presence or absence or quantifying the amount of said one or more synthesized nucleic acid molecules by measuring said one or more detectable labels.

12. (Currently amended) A method for quantitation or detection of one or more target nucleic acid molecules in a sample during nucleic acid amplification comprising:

mixing one or more target nucleic acid molecules with one or more detectably labeled oligonucleotides under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said one or more target nucleic acid molecules, said one or more amplified nucleic acid molecules comprising said one or more oligonucleotides, wherein said one or more oligonucleotides comprise one or more detectable labels located only internally and said one or more labels undergo a detectable change in an observable property upon ~~hybridizing~~ hybridization of said one or more

oligonucleotides to said one or more target nucleic acid molecules; wherein said detectable change in ~~an observable property~~ is not the result of ~~a transfer of energy between two different compounds attached to said one or more oligonucleotides~~ fluorescence resonance energy transfer (FRET); and

detecting the presence or absence or quantifying the amount of said one or more target nucleic acid molecules by measuring the said detectable labels of said oligonucleotides.

13. (Currently amended) The method of claim 10, 11 or 12, wherein said label is selected from the group consisting of fluorescent labels, chemiluminescent labels and bioluminescent labels.

14. (Original) The method of claims 11 or 12, wherein said detection step comprises detecting or measuring the level of activity of the detectable label during said synthesis or amplification compared to the level of activity of the detectable label in the absence of said synthesis or amplification.

15. (Original) The method of claim 12, wherein said amplification is accomplished by at least one method selected from the group consisting of PCR, 5-RACE, RT PCR, Allele-specific PCR, Anchor PCR, "one-sided PCR," LCR, NASBA, and SDA.

16. (Original) The method of claim 13, wherein said oligonucleotides comprise one or more fluorescent labels.

17. (Original) The method of anyone of claims 10, 11 or 12, wherein said one or more oligonucleotides comprise one or more hairpin structures.

18. (Currently amended) A method for amplifying a double stranded nucleic acid molecule, comprising:

providing a first and second primer, wherein said first primer is complementary to a sequence within or at or near the 3'-termini of the first strand of said nucleic acid molecule and said second primer is complementary to a sequence within or at or near the 3'-termini of the second strand of said nucleic acid molecule;

hybridizing said first primer to said first strand and said second primer to said second strand in the presence of one or more polymerases, under conditions such that a third nucleic acid molecule complementary to all or a portion of said first strand and a fourth nucleic acid molecule complementary to all or a portion said second strand are synthesized;

denaturing said first and third strands, and said second and fourth strands; and repeating the above steps one or more times, wherein one or more of the primers is labeled with ~~comprise one or more~~ detectable labels located only internally;

~~with the proviso that said one or more detectably labeled oligonucleotides do not comprise an acceptor molecule~~

wherein said one or more labels undergo a detectable change in an observable property upon ~~hybridizing~~ hybridization of said one or more labeled primers to said nucleic acid molecule, and wherein said detectable change ~~in an observable property~~ is not the result of ~~a transfer of energy between two different compounds attached to said one or more primers~~ fluorescence resonance energy transfer (FRET).

19. (Original) The method of claim 18, wherein at least one of said primers comprises at least one hairpin structure.

20. (Currently amended) A method for the quantification or detection of nucleic acid molecules comprising:

mixing one or more labeled oligonucleotides with one or more target nucleic acid molecules to be detected or quantitated, wherein said one or more oligonucleotides comprise one or more fluorescent labels located only internally; wherein said one or more labels undergo a detectable change in an observable property upon ~~hybridizing~~ hybridization of said one or more oligonucleotides to said one or more target nucleic acid molecules, and wherein said detectable change ~~in an observable property~~ is not the result of ~~a transfer of energy between two different compounds attached to said one or more oligonucleotides~~ fluorescence resonance energy transfer (FRET); and

detecting or measuring an increase in fluorescence associated with said one or more oligonucleotides hybridizing to said one or more target nucleic acid molecules.

21. (Original) The method of claim 20, wherein the fluorescent label is FAM.

22. (Original) The method of claim 20, wherein the fluorescent label is TAMRA.

23 - 46. (Cancelled)

47. (Currently amended) A method for detecting a target nucleic acid sequence, comprising:

contacting a sample containing a mixture of nucleic acid molecules with one or more oligonucleotides which comprise one or more detectable labels located only internally and are capable of hybridizing a target nucleic acid molecule, wherein said one or more detectable labels undergo a change in one or more observable properties upon hybridization ~~to the target nucleic acid molecule~~ said hybridizing; wherein said change ~~in an observable property~~ is not the result of a ~~transfer of energy between two different compounds attached to said one or more oligonucleotides~~ fluorescence resonance energy transfer (FRET); and

observing the observable property, wherein a change in the observable property indicates the presence of the target nucleic acid sequence.

48 - 55. (Cancelled)

56. (Previously presented) The method of claim 10, wherein said one or more oligonucleotides further comprise one or more detectable labels at or near their 3' and/or 5' termini.

57. (Previously presented) The method of claim 11, wherein said one or more oligonucleotides further comprise one or more detectable labels at or near their 3' and/or 5' termini.

58. (Previously presented) The method of claim 12, wherein said one or more oligonucleotides further comprise one or more detectable labels at or near their 3' and/or 5' termini.

59. (Previously presented) The method of claim 18, wherein said primers further comprise one or more hairpin structures.

60. (Previously presented) The method of claims 18 or 59, wherein said one or more primers further comprise a detectable label at or near their 3' and/or 5' termini.

61. (Previously presented) The method of claim 20, wherein said one or more oligonucleotides further comprise one or more detectable labels at or near their 3' and/or 5' termini.

62. (Previously presented) The method of claim 47, wherein said one or more oligonucleotides further comprise one or more detectable labels at or near their 3' and/or 5' termini.

63. (Previously presented) The method of any one of claims 10, 11, 12, 18, 20 or 47, wherein said detectable label is at the fourth base from the 3' terminus.

64. (Previously presented) The method of any one of claims 10, 11, 12, 18, 20 or 47, wherein said detectable label is at the fifth base from the 3' terminus.

65. (Previously presented) The method of any one of claims 10, 11, 12, 18, 20 or 47, wherein said detectable label is at the sixth base from the 3' terminus.

66. (Previously presented) The method of any one of claims 10, 11, 12, 18, 20 or 47, wherein said detectable label is attached to one of the ten 3'-most terminal nucleotides.

67. (Previously presented) The method of any one of claims 10, 11, 12, 18, 20 or 47, wherein said detectable label is attached to one of the twenty 3'-most terminal nucleotides.

68 - 73. (Cancelled)

74. (Currently amended) The method of any one of claims 10-12, 18, 20, and ~~47, and 68-73~~, wherein said oligonucleotides comprise a single label.

75. (Previously presented) The method of claim 74, wherein said single label is a fluorescent label.

76. (New) The method of claim 20, wherein said fluorescent label is 2'7'-dimethoxy-4'5'-dichloro-6-carboxyfluorescein (JOE).

77. (New) The method of claim 20, wherein said fluorescent label is 6-carboxy-X-rhodamine (ROX).